gp100 gene. This fragment was digested with *Eco*RV and *Xho*I and cloned into *Eco*RV/*Xho*I digested NVQH6MC5#10 generating plasmid C5H6MELgp100 #5 which contains the gp100 gene linked to the H6 promoter.

The gp100 gene in plasmid C5H6MELgp100 #5 was sequenced using custom primers. A 65bp deletion was found in this clone and shown to be present in pCDNA3-gp100. Plasmid PCRII-gp100 was used in PCR with oligonucleotides MELgp05(5'-CCC-ATC-TGG-CTC-TTG-GTC-3') (SEQ.ID.NO. 115) and MELgp13 (5'-TGA-CAT-CTC-TGC-CAG-TGT-GGT-3') (SEQ.ID.NO. 116) to generate a 0.6kb fragment. This fragment was digested with *Bam*HI and *Asp*718 and ligated to a 6.5kb *Asp*718/*Bam*HI (partial) fragment from C5H6MELgp100 #5 generating plasmid C5H6MELgp100 which contains the entire gp100 gene under the control of the H6 promoter.

Pre-existing plasmid pC5H6MELgp100 was used as template for site directed mutagenesis of the two CTL epitopes beginning at amino acids 209 and 280, respectively. Primers used were:

209-A

GCT CAG CCT TCA CCA TTA TGG ACC AGG TGC CTT TCT CC (SEQ.ID.NO.117)

209-B

GGA GAA AGG CAC CTG GTC CAT AAT GGT GAA GGC TGA CG (SEQ.ID.NO.118)

280-A

GAG CCT GGC CCA GTC ACT GTT CAG GTG GTC CTG CAG GC (SEQ.ID.NO.119)

280-B

GCC TGC AGG ACC ACC TGA ACA GTG ACT GGG CCA GGC TC (SEQ.ID.NO.120)

A section containing the modified epitopes was sequenced and isolated as a 440 bp *Nco*1/*Mlu*N1 fragment. This fragment was ligated into

pC5H6MELgp100 digested with *Nco*1 and *Mlu*N1, creating a plasmid with the complete gp100 with the modified epitopes 209-2M and 280-9V.

Sequence data revealed a G to C substitution at bp# 10, changing a.a. # 4 from a Valine to a Leucine. This was corrected by PCR using the following primer pair;

MEL25

GCT CCG GGA TCC CCG GCG ATG GTA GAC AGT CAC TTC CAT CGT GTG TGT GCC CAG CAT TG (SEQ.ID.NO.121)

MEL27

ATC GCG ATA TCC GTT AAG TTT GTA TCG TAA TGG ATC TGG TGC TAA AAA GAT GCC TTC TT (SEQ.ID.NO.122)

MEL25 changes bp# 549 from a C to a G destroying the unique *Nco*1 site for easier screening. It does not change the amino acid.

The resulting PCR fragment was digested with *Bam*H1 and *Eco*R5 and replaced the equivalent fragment correcting the error. The resulting plasmid is pC5gp100-M which is shown in Figure 3 (SEQ.ID.NO.123).

<u>Genetic modification of the recipient:</u>

Recombination between donor plasmid pC5gp100M and ALVAC(2) rescuing virus generated recombinant virus vCP1584, which contains the vaccinia H6 promoted modified human gp100 in the C5 locus.

<u>EXAMPLE 3</u>

<u>Screening for the identification and purification of recombinant organisms:</u>

The aspects of screening for the identification and purification of a recombinant organism of the present invention is set out below.

(1) <u>Plaque purification</u> was done using *in situ* plaque hybridization (Piccini *et al.*, Methods of Enzymol. 153:545 (1987)) was used to identify recombinant viruses and to demonstrate purity of final virus preparations. *In situ* plaque hybridization analysis was performed with radiolabelled probes specific for the gp100 construct (a 580 bp fragment) and the C5 insertion locus.

(2) Restriction analysis: Viral genomic DNA was isolated from cells infected with ALVAC parent or ALVAC(2)-gp100M (vCP1584). The genomic DNA was digested with restriction endonucleases (*Hind*III, *Pst* I or *Bam*HI). The resultant DNA fragments were fractionated by electrophoresis through an agarose gel and visualized by ethidium bromide staining. The insertion of the mod gp100 expression cassette at the C5 locus was confirmed.

(3) Immunoprecipitation analyses: These were performed using radiolabeled lysates derived from uninfected HeLa cells or cells infected with either ALVAC parental virus, ALVAC-gp100 (vCP1465) or ALVAC(2)-gp100M (vCP1584) as described previously (Taylor *et al* . J. Virol. 64:1441 (1990)). Briefly, HeLa cell cultures were infected at an m.o.i. of 10 pfu/cell in methionine-free media supplemented with [35S]-methionine (35uCi/ml). At 18 hrs. post infection, cells were lysed. Immunoprecipitation was performed using a rabbit anti-gp100 serum (AZN-LAM, received from M. Schreurs University of Nijmegen, Netherlands). Immunoprecipitates were fractionated on a 10% SDS-Polyacrylamide gel. The gel was fixed and treated for fluorography with 1M Na-salicylate for 1/2 hr. The dried gel was exposed to Kodak XAR-2 film to visualize the protein species. Results with anti-gp100 demonstrate expression of gp100 in ALVAC-gp100 infected HeLa cells but not for parentally infected cells. (See Figure 6)

(4) Western Blot. HeLa cells were infected for 18 hours at a multiplicity of 10 pfu/cell with ALVAC(2)-gp100M (vCP1584), ALVAC-gp100 (vCP1465) or ALVAC. Cell lysates were separated by SDS-PAGE and transferred to nitrocellulose. The blot was incubated with AZN-LAM (1/5000 dilution) followed by HRP conjugated swine anti-rabbit utilizing the enhanced chemiluminescence (ECL) detection method (Amersham). Results demonstrate expression of full length gp100 in ALVAC-gp100 and ALVAC(2)-gp100M infected cells. (See Figure 7).

(5) Plaque immunoscreen analysis. This was performed on vCP1584 material to determine phenotypic stability of the virus upon passaging. The

phenotypic stability of production batch material of ALVAC-gp100M (vCP1584) was analyzed by an immunlogical plaque assay which measures expression of the inserted genes at the plaque level. The assay utilizing permeabilized cells for detection of intracellular as well as surface expression of Hgp100mod was chosen for this test.

Test and control reagents (ALVAC(2)-gp100M (vCP1584) and ALVAC standard and ALVAC-gp100M, respectively) were plated on CEF monolayers under agarose at dilutions resulting in 40-200 plaques per 60 mm dish. 120 hours after incubation at 37°C, the infected monolayers were processed by plaque immunoassay for detection of internal expression of gp100M. Positive and negative plaques were counted for test and control samples. The primary antibody used was Monoclonal Anti-HMB50 at 1:800 dilution. A secondary antibody used was horse radish peroxidase (HRP)-conjugated rabbit anti-mouse antiserum diluted 1:500.

The result of analysis of internal expression of Human modified gp100 by individual plaques produced by (vCP1584) is presented in Table 1.

The result demonstrates that 98.7% of the plaque population of ALVAC-gp100M is expressing gp100M indicating that ALVAC-gp100M is phenotypically stable.

Results of the plaque immunoscreen analysis demonstrate that ALVAC(2)-gp100M is phenotypically stable with respect to expression of gp100.

(6) Nucleotide sequence analysis. This was performed on vCP1584 to validate the nucleotide sequence of the H6-promoted melanoma gp100M cassette. The sequence analysis revealed no nucleotide differences relative to the expected sequence, thus no mutations were introducted during the production of vCP1584. In order to carry out this analysis, a pool of plasmid clones containing a 2.2 kb PCR-derived fragment (encompassing the H6-promoted melanoma gp100M cassette), generated from vCP1584 genomic DNA was used.

pBS/1584 was generated by pooling 9 positive clones obtained by the ligation of a 2.2 kb PCR fragment (containing the H6-promoted melanoma

gp100M cassette from vCP1584), into pBS-sk-(Stratogene). The 2.2 kb PCR fragment was derived from vCP1584 genomic DNA with the oligonucleotide primers, IDC5-1 and IDC5-2 (Figure 5). The nucleotide sequence of the oligonucleotide primers used to sequence pBS/1584 are listed in Figure 5.

EXAMPLE 4

This example provides results from injection in cynomolgus monkeys of modified gp100 molecules.

Methods and Experimental Design

Test System

Cynomolgus monkeys (Macaca fascicularis) purpose bred animals.

Supplier: Siconbrec "Simian Conservation Breeding & Research Center Inc.", Fema Building, 44 Gil Puyat Avenue Makati, Metro Manila, Philippines.

Number of animals in the study: 12 (6 males and 6 females).

Age at initiation of treatment: 26 to 38 months.

- Body weight range at initiation of treatment (day -1):
- males: 1.73 to 2.34 kg
- females: 1.71 to 2.65 kg.

Animal Husbandry

- Housing: one air-conditioned room;
- temperature: 19 to 25°C (target range),
- relative humidity: >40%
- air changes: minimum 8 air changes per hour,
- lighting cycle: 12 hours light (artificial)/12 hours dark.
- Caging: animals were housed singly in stainless steel mesh cages (approximately 540 x 810 x 760 mm).
- Diet: expanded complete commercial primate diet (Mazuri diet, Special Diet Services Ltd., Witham, Essex, CM8, 3AD, Great Britain) analyzed for chemical and bacterial contaminants.

Quantity distributed: 100g diet/animal/day.

In addition, animals received fruit daily (apple or banana)

Animals were fasted for at least 16 hours before blood sampling for clinical laboratory investigations and before necropsy.

- Water: drinking water *ad libitum* (via bottles).

- Contaminants: no known contaminants were present in diet or water at levels which might have interfered with achieving the objective of the study.

Pre-Treatment Procedures

- Animal health procedure: all animals received a clinical examination for ill-health on arrival and a veterinary clinical examination during the acclimatization period.

- Acclimatization period: at least 3 weeks between animal arrival and start of treatment.

Experimental Design

- Allocation to treatment groups was performed during the acclimatization period using a random allocation procedure based on body weight classes.

- Animals were assigned to the treatment groups shown in Table 2. The dose levels administered were shown in Table 3.

Administration of the Test/Control Articles

Group 1 and 2 Animals

- Method of administration: injection in the left inguinal lymph node. Animals were lightly anaesthetized before each administration by an intramuscular injection of ketmine hydrochloride (Imalgene® 500 - Merial, Lyon, France). The same lymph node was injected on each occasion (left side). Each injection was followed by a local disinfection with iodine (Vétédine® - Vétoquinol, Lure, France).

Group 3

- Route: subcutaneous.

- Method of administration: bolus injection using a sterile syringe and needle introduced subcutaneously. Four injection sites were used followed by a local disinfection with iodine (Vétédine® - Vétoquinol, Lure, France). Animals were also lightly anaesthetized before each administration by an intramuscular

injection of ketamine hydrochloride (Imalgene® 500 - Merial, Lyon, France) in order to be under the same conditions as groups 1 and 2 animals.

Four injection sites in the dorsal cervical/interscapular regions were used as shown in Table 4.

ELISPOT Analysis

An ELISPOT assay was used in order to assess the cell mediated immune response generated in the monkeys in the various treatment groups. In particular, an ELISPOT IFNγ assay was used in order to measure IFNγ production from T lymphocytes obtained from the monkeys in response to gp100 antigens.

Materials and Methods

Plates: MILLIPORE Multiscreen HA plate / MAHA S45.10 (96 wells).

Capture antibodies: MABTECH monoclonal anti-IFNγ antibodies/G-Z4 1 mg/mL.

Detection antibodies: MABTECH monoclonal anti-IFNγ antibodies/7-B6-1-biotin 1 mg/mL.

Enzyme: SIGMA, Extravidin-PA conjuate/E2636

Substrate: BIORAD, NBT/BCIP - Alkaline phosphatase conjugate substrate kit/ref: 170-64 32.

Coating

Place 100 µL per well of capture antibodies at 1 µg/mL diluted at 1/1000 in carbonate bicarbonate buffer 0.1M pH 9.6 into the multiwell plate. Incubate overnight at 4°C. Wash 4 times in 1X PBS.

Saturation

Place 200 µL per well of RPMI supplemented with 10% FCS, non essential amino acids, pyruvate, Hepes buffer and Peni-Strepto. Incubate 2 hours at 37°C.

Test

Cells from the immunized animals are tested against (a) medium alone; (b) pooled peptides at a concentration of 1 mg/mL; and (c) a non specific stimulus (PMA-Iono). The pooled peptides used in this Example to stimulate IFN-γ

production were derived from gp100 and are illustrated in Tables 5 to 8. The final volume of each sample is 200 µL. Incubate 20 hours at 37°C.

Wash 4 times in 1X PBS and 0.05% Tween 20.

Detection

Place 100 µL per well of detection antibodies at 1 µg/mL diluted in 1/1000 1X PBS, 1% BSA and 0.05% Tween 20. Incubate 2 hours at room temperature. Wash 4 times in 1X PBS and 0.05% Tween 20.

Reaction

Place 100 µL per well of Extravidin-PA conjugate diluted 1/6000 in 1X PBS, 1% BSA and 0.05% Tween 20. Incubate 45 minutes at room temperature. Wash 4 times in 1X PBS and 0.05% Tween 20.

Substrate Addition

Place 100 µL per well of substrate previously prepared. For example, for 1 plate, prepare: 9.6 mL of distilled water, 0.4 mL of 25X buffer, 0.1 mL of solution A (NBT) and 0.1 mL of solution B (BCIP). Incubate 30-45 minutes at room temperature. Wash in distilled water. Dry and transfer to a plastic film. The number of spots are counted using a Zeiss image analyzer. Each spot corresponds to an individual IFN-γ secreting T cell.

Results

The results of the ELISPOT analysis are shown in Figures 8-11. The results demonstrate that of the animals tested, 2 out of 2 (i.e. 100%) of the animals that received the intranodal administration of the gp100 antigen, and 2 out of 4 (i.e. 50%) of the animals that received the subcutaneous administration of the gp100 antigen had a positive cell mediated immune response.

ELISA Analysis

The ELISA was performed utilizing standard methodology known in the art. Briefly, the human gp100 ("hgp100"; produced in Baculovirus) was diluted in coating buffer (carbonate-bicarbonate, pH9.6) and added to 96 wells at 0.5ug/well. Plates were placed at 4°C overnight. Plates were then washed and blocking buffer (phosphate buffered saline/0.5% Tween 20/1.0% BSA, pH7.2)

was added for 2 hours at 37°C. The plates were then washed and the sera was diluted in dilution buffer (phosphate buffered saline/0.5 % Tween 20/ 0.1 BSA, pH7.2). For this study, monkey sera was diluted to 1:800 and "7" serial 3 fold dilutions were done for each sample tested. The human sera controls were diluted to 1:50 in dilution buffer and "7" serial 2 fold dilutions were performed. Each dilution was done in duplicate. The plates were incubated a further 2 hours at 37°C. The plates were washed and the horse radish peroxidase (HRP)-conjugated anti-human secondary antibody (anti-human Ig whole antibody from sheep (Amersham Life Science, NA933)) diluted 1:100 in dilution buffer was added to the wells and incubated for 1 hour at 37°C. The plates were washed and OPD (o-phenylenediamine dihydrochloride) substrate with H_2O_2 in substrate buffer (50mM phosphate/25mM citrate, pH 7.2) was added to the wells. For a kinetics ELISA, the plate was read repeatedly (2 minute intervals for 15 minutes) unstopped (without "stop" buffer). Plates were read at 450mm.

Results

The results of the above experiment are presented in Table 9 and in Figure 12. The animals of group 2 received intranodal injections of ALVAC(2)-gp100(mod) followed by boosts with the modified gp100 peptides 209(2M) and 290(9V); the animals in group 3 received a subcutaneous injection of the ALVAC(2) construct followed by peptide boosts; the animals in group 1 received intranodal injections of saline as a control.

As can be seen from Figure 12, both types of injection of the antigens induced a significant humoral response to the antigen.

In summary, the results of this Example demonstrate that injection of a tumor antigen accoding to the invention induces both a significant humoral and cell mediated response.

EXAMPLE 5

This example presents data obtained from human melanoma patients primed with ALVAC(2)-gp100M and boosted with modified gp100 peptides (g209-2M and g280-9V).

Immunization Protocol

5 Patients were immunized subcutaneously in a prime-boost schedule with ALVAC(2)-gp100M ("prime"; lyophilzed ALVAC(2)-gp100M resuspended in 1 ml of 0.4% NaCl; 0.5 ml injections (approximately $0.5 \times 10^{7.09}$ $CCID_{50}$ per injection)) and peptides g209-2M and g280-9V ("boost"; 1000µg/peptide in 1 ml total volume per week (0.2 ml/injection per day x 5 days)). All patients: 1) were HLA-A0201 positive; 2) were between 18 and 70 years of age; 3) exhibited pathologically confirmed malignant melanoma; 4) demonstrated immunocompetence by reactivity to at least 2 or more out of 7 Cell Mediated Immunity (CMI) skin tests; 5) had blood hematology and chemistry values within the following ranges:

I) Hematology:

Hemoglobin	>	100g/L
Granulocytes	>	2.0×10^9/L
Lymphocytes	>	1.5×10^9/L
Platelets	>	100×10^9/L

II) Chemistry:

Serum creatinine	< 150 _mol/L
Serum total bilrubin	<30 _mol/L
AST, ALT, and ALP	Must be < 2x the normal upper limit or <5x the normal upper limit if due to liver metastases.

Patients "primed" with ALVAC(2)-gp100M on weeks 1, 4 and 7; "boosted" with peptides on weeks 10 and 13.

ELISPOT Analysis: These results are present in Tables 10 and 11. Peripheral Blood Mononuclear Cells ("PBMNC") were isolated by density centrifugation

over Ficoll gradients. Cells were bulk-cultured at $3x10^6$/ml in AIM-V media along with a mixture of g209-2M and g280-9V or the HLA-A*0201 binding Flu peptide (all at 50 μg/ml) for 8 days. IL-2 was added on days 3 and 5 of culture. On day 9, cells were harvested, counted and $2x10^5$ cells/well plus 50 U/ml IL-2, with and without the respective peptides, were plated in nitrocellulose membrane containing ELISPOT plates that had been precoated with anti-INF-γ antibodies. The plates were developed after 48 hours of culture. The numbers reported are the differences between the average of two wells restimulated with peptide and IL-2 and two wells treated only with IL-2.

Responses are the number of spots (counted by the electronic ELISPOT reader but confirmed in most cases by manual counting) per $2x10^5$ PBMNC. The number of CD8+ T cells was not routinely determined but is typically 2-5-fold less than this number.

Having illustrated and described the principles of the invention in a preferred embodiment, it should be appreciated by those skilled in the art that the invention can be modified in arrangement and detail without departure from such principles. We claim all modifications coming within the scope of the following claims.

All publications, patents and patent applications referred to herein, are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

TABLE 1

Analysis of expression of gp100 antigen by ALVAC-gp100M

	Human gp100M			
	Positive Plaques	negative plaques	total # of plaques	% positive
ALVAC std.	0	571	571	0
vCP1584	387	0	387	100
ALVAC gp100mod L	875	11	886	98.7

TABLE 2

Group Number	Route of administration	Treatment days and compound administered	Number of Animals
1	Intranodal	Saline (NaCl 0.9%): days 28, 42, 56 Then 70, 71, 72, 73, 74 Then 84, 85, 86, 87 and 88	4
2	Intranodal	ALVAC(2) - gp100 mod: days 28, 42, 56 *mgp100 peptides: days 70, 71, 72, 73, 74 Then 84, 85, 86, 87 and 88	4
3	Subcutaneous	Saline (NaCl 0.9%): day 1 ALVAC(2) - gp100 mod: days 28, 42, 56 *mgp100 peptides: days 70 and 84	4

*209(2M)-IMDQVPFSY (SEQ.ID.NO.124); 290(9V) YLEPGPVTV (SEQ.ID.NO.125)

- Group 1 animals (control) received the control article (saline for injection (NaCl 0.9%)).
- Group 3 animals received the control article (saline for injection (NaCl 0.9%)) on day 1 only.

TABLE 3

Group Number	Dose level	Dose volume (ml/administration)
1	Saline (NaCl 0.9%): 0	0.250
2	Dose: 0.25 x $10^{7.4}$ CCID 50 ALVAC (2) - gp100 mod: 0.25 $10^{7.4}$ CCID50	0.250
	Dose: 200 µg (Total) of peptides IMDQVPFSY (209(2M)), and YLEPGPVTV (290(9V)) (100µg each)	0.2
3	Saline (NaCl 0.9%)	0.250
	ALVAC(2) - gp100 mod: 0.25 $10^{7.4}$ CCID 50	0.250
	Dose: 200 µg (Total) of peptides IMDQVPFSY (209(2M)), and YLEPGPVTV (290(9V)) (100µg each)	0.2

TABLE 4

Days	Sites used
1 and 28	lower left
42	upper left
56	upper right
70	lower left
84	lower right

TABLE 5

Peptide Pool #1

Peptide	Sequence	SEQ.ID.NO.
1329	HLAVIGALLAVGATK	SEQ.ID.NO.3
1330	GALLAVGATKVPRNQ	SEQ.ID.NO.4
1331	VGATKVPRNQDWLGV	SEQ.ID.NO.5
1332	VPRNQDWLGVSRQLR	SEQ.ID.NO.6
1333	DWLGVSRQLRTKAWN	SEQ.ID.NO.7
1334	SRQLRTKAWNRQLYP	SEQ.ID.NO.8
1335	TKAWNRQLYPEWTEA	SEQ.ID.NO.9
1336	RQLYPEWTEAQRLDC	SEQ.ID.NO.10
1337	EWTEAQRLDCWRGGQ	SEQ.ID.NO.11
1338	QRLDCWRGGQVSLKV	SEQ.ID.NO.12
1339	WRGGQVSLKVSNDGP	SEQ.ID.NO.13
1340	VSLKVSNDGPTLIGA	SEQ.ID.NO.14
1344	IALNFPGSQKVLPDG	SEQ.ID.NO.15
1345	PGSQKVLPDGQVIWV	SEQ.ID.NO.16
1346	VLPDGQVIWVNNTII	SEQ.ID.NO.17
1347	QVIWVNNTIINGSQV	SEQ.ID.NO.18
1348	NNTIINGSQVWGGQP	SEQ.ID.NO.19
1349	NGSQVWGGQPVYPQE	SEQ.ID.NO.20
1350	WGGQPVYPQETDDAC	SEQ.ID.NO.21
1351	VYPQETDDACIFPDG	SEQ.ID.NO.22
1352	TDDACIFPDGGPCPS	SEQ.ID.NO.23
1353	IFPDGGPCPSGSWSQ	SEQ.ID.NO.24
1355	GSWSQKRSFVYVWKT	SEQ.ID.NO.25
1356	KRSFVYVWKTWGQYW	SEQ.ID.NO.26
1357	YVWKTWGQYWQVLGG	SEQ.ID.NO.27
1358	WGQYWQVLGGPVSGL	SEQ.ID.NO.28
1359	QVLGGPVSGLSIGTG	SEQ.ID.NO.29

TABLE 6

Peptide Pool #2

Peptide	Sequence	SEQ.ID.NO.
1360	PVSGLSIGTGRAMLG	SEQ.ID.NO.30
1361	SIGTGRAMLGTHTME	SEQ.ID.NO.31
1362	RAMLGTHTMEVTVYH	SEQ.ID.NO.32
1363	THTMEVTVYHRRGSR	SEQ.ID.NO.33
1364	VTVYHRRGSRSYVPL	SEQ.ID.NO.34
1365	RRGSRSYVPLAHSSS	SEQ.ID.NO.35
1366	SYVPLAHSSSAFTIT	SEQ.ID.NO.36
1368	AFTITDQVPFSVSVS	SEQ.ID.NO.37
1369	DQVPFSVSVSQLRAL	SEQ.ID.NO.38
1370	SVSVSQLRALDGGNK	SEQ.ID.NO.39
1372	DGGNKHFLRNQPLTF	SEQ.ID.NO.40
1373	HFLRNQPLTFALQLH	SEQ.ID.NO.41
1374	QPLTFALQLHDPSGY	SEQ.ID.NO.42
1375	ALQLHDPSGYLAEAD	SEQ.ID.NO.43
1379	DFGDSSGTLISRALV	SEQ.ID.NO.44
1380	STGLISRALVVTHTY	SEQ.ID.NO.45
1381	SRALVVTHTYLEPGP	SEQ.ID.NO.46
1382	VTHTYLEPGPVTAQV	SEQ.ID.NO.47
1383	LEPGPVTAQVVLQAA	SEQ.ID.NO.48
1384	VTAQVVLQAAIPLTS	SEQ.ID.NO.49
1385	VLQAAIPLTSCGSSP	SEQ.ID.NO.50
1386	IPLTSCGSSPVPGTT	SEQ.ID.NO.51
1388	VPGTTDGHRPTAEAP	SEQ.ID.NO.52
1389	DGHRPTAEAPNTTAG	SEQ.ID.NO.53
1390	TAEAPNTTAGQVPTT	SEQ.ID.NO.54
1392	QVPTTEVVGTTPGQA	SEQ.ID.NO.55
1393	EVVGTTPGQAPTAEP	SEQ.ID.NO.56

TABLE 7

Peptide Pool #3

Peptide	Sequence	SEQ.ID.NO.
1394	TPGQAPTAEPSGTTS	SEQ.ID.NO.57
1395	PTAEPSGTTSVQVPT	SEQ.ID.NO.58
1396	SGTTSVQVPTTEVIS	SEQ.ID.NO.59
1397	VQVPTTEVISTAPVQ	SEQ.ID.NO.60
1398	TEVISTAPVQMPTAE	SEQ.ID.NO.61
1399	TAPVQMPTAESTGMT	SEQ.ID.NO.62
1400	MPTAESTGMTPEKVP	SEQ.ID.NO.63
1401	STGMTPEKVPVSEVM	SEQ.ID.NO.64
1402	PEKVPVSEVMGTTLA	SEQ.ID.NO.65
1403	VSEVMGTTLAEMSTP	SEQ.ID.NO.66
1404	GTTLAEMSTPEATGM	SEQ.ID.NO.67
1405	EMSTPEATGMTPAEV	SEQ.ID.NO.68
1408	SIVVLSGTTAAQVTT	SEQ.ID.NO.69
1409	SGTTAAQVTTTEWVE	SEQ.ID.NO.70
1410	AQVTTTEWVETTARE	SEQ.ID.NO.71
1411	TEWVETTARELPIPE	SEQ.ID.NO.72
1412	TTARELPIPEPEGPD	SEQ.ID.NO.73
1413	LPIPEPEGPDASSIM	SEQ.ID.NO.74
1414	PEGPDASSIMSTESI	SEQ.ID.NO.75
1415	ASSIMSTESITGSLG	SEQ.ID.NO.76
1416	STESITGSLGPLLDG	SEQ.ID.NO.77
1417	TGSLGPLLDGTATLR	SEQ.ID.NO.78
1418	PLLDGTATLRLVKRQ	SEQ.ID.NO.79
1419	TATLRLVKRQVPLDC	SEQ.ID.NO.80
1420	LVKRQVPLDCVLYRY	SEQ.ID.NO.81
1421	VPLDCVLYRYGSFSV	SEQ.ID.NO.82
1422	VLYRYGSFSVTLDIV	SEQ.ID.NO.83

Table 8

Peptide Pool #4

Peptide	Sequence	SEQ.ID.NO.
1424	TLDIVQGIESAEILQ	SEQ.ID.NO.84
1425	QGIESAEILQAVPSG	SEQ.ID.NO.85
1426	AEILQAVPSGEGDAF	SEQ.ID.NO.86
1427	AVPSGEGDAFELTVS	SEQ.ID.NO.87
1428	EGDAFELTVSCQGGL	SEQ.ID.NO.88
1429	ELTVSCQGGLPKEAC	SEQ.ID.NO.89
1430	CQGGLPKEACMEISS	SEQ.ID.NO.90
1431	PKEACMEISSPGCQP	SEQ.ID.NO.91
1432	MEISSPGCQPPAQRL	SEQ.ID.NO.92
1434	PAQRLCQPVLPSPAC	SEQ.ID.NO.93
1435	CQPVLPSPACQLVLH	SEQ.ID.NO.94
1436	PSPACQLVLHQILKG	SEQ.ID.NO.95
1437	QLVLHQILKGGSGTY	SEQ.ID.NO.96
1441	LADTNSLAVVSTQLI	SEQ.ID.NO.97
1442	SLAVVSTQLIMPGQE	SEQ.ID.NO.98
1443	STQLIMPGQEAGLGQ	SEQ.ID.NO.99
1444	MPGQEAGLGQVPLIV	SEQ.ID.NO.100
1445	AGLGQVPLIVGILLV	SEQ.ID.NO.101
1448	LMAVVLASLIYRRRL	SEQ.ID.NO.102
1450	YRRRLMKQDFSVPQL	SEQ.ID.NO.103
1451	MKQDFSVPQLPHSSS	SEQ.ID.NO.104
1452	SVPQLPHSSSHWLRL	SEQ.ID.NO.105
1453	PHSSSHWLRLPRIFC	SEQ.ID.NO.106
1454	HWLRLPRIFCSCPIG	SEQ.ID.NO.107
1455	PRIFCSCPIGENSPL	SEQ.ID.NO.108

TABLE 9

	DAY (mOD/min)			
Monkey #	0	57	68	96
1	3	5	2	2
2	4	6	12	10
3	7	6	10	8
4	7	6	8	8
5	5	9	20	15
6	11	8	10	12
7	11	23	51	30
8	7	30	70	22
9	1	7	5	3
10	2	6	6	4
11	3	7	14	8
12	6	9	15	6

TABLE 10

Gp100-specific responses to g209-2M and g280-9V*

Patient	Pre 1st Injection	Pre 2nd Injection	Pre 3rd Injection	Pre 4th Injection	Pre 5th Injection	4 wks post vaccination
#1	0	0	0	ND	ND	2±1.4
#2	0	14±2.8	54±6.4	16±7.8	ND	ND
#3	0	0	ND	ND	ND	ND
#4	0	0	24±13.4	1±2.1	ND	ND
#5	ND	6±6.4	ND	ND	ND	ND

TABLE 11

Flu-peptide specific responses*

Patient	Pre 1st Injection	Pre 2nd Injection	Pre 3rd Injection	Pre 4th Injection	Pre 5th Injection	4 wks post vaccination
#1	>150	ND	>70	ND	ND	12.5
#2	ND	0	24	0	ND	ND
#3	23.5	7	ND	ND	ND	ND
#4	0	29	13.5	11.5	ND	ND
#5	ND	>200	ND	ND	ND	ND

* ND signifies that the values were not determined for the sample.

WE CLAIM:

1. An isolated and purified modified gp100 molecule capable of modulating an immune response in an animal.

2. A molecule according to claim 1 having a nucleic acid sequence shown in Figure 1 (SEQ.ID.NO.1).

3. A molecule according to claim 1 or 2 which comprises:

(a) a nucleic acid sequence as shown in Figure 1 (SEQ.ID.NO.1) wherein T can also be U;

(b) nucleic acid sequences complementary to (a);

(c) nucleic acid sequences which are homologous to (a) or (b);

(d) a fragment of (a) to (c);

(e) a nucleic acid which will hybridize to (a) to (d) under stringent hybridization conditions; and

(f) a nucleic acid molecule differing from any of the nucleic acids of (a) to (d) in codon sequences due to the degeneracy of the genetic code.

4. The nucleic acid of any one of claims 1-3 wherein the nucleic acid is selected from the group consisting of viral nucleic acid, plasmid, bacterial DNA, naked/free DNA, and RNA.

5. A viral nucleic acid of claim 4 wherein the virus is selected from adenovirus, alphavirus or poxvirus.

6. A poxvirus of claim 5 which is vaccinia, fowlpox, avipox, TROVAC, ALVAC, NYVAC or MVA.

7. The poxvirus of claim 6 which is ALVAC.

8. A composition comprising the nucleic acid of any one of claims 1-7 and a pharmaceutically acceptable diluent or carrier.

9. A composition according to claim 8 further comprising an adjuvant.

10. A cell comprising a nucleic acid according to any one of claims 1-7 wherein the cell expresses a polypeptide encoded by the nucleic acid.

11. A cell according to claim 10 wherein the cell is an antigen-presenting cell.

12. A cell according to claim 10 wherein the cell is a dendritic cell.

13. A recombinant virus comprising a virus into which is inserted a nucleic acid according to any one of claims 1-7 wherein the nucleic acid encodes for a polypeptide, the recombinant virus causing the expression of the polypeptide in an infected cell.

14. A recombinant virus into which is inserted a nucleic acid according to any one of claims 1-7 wherein the nucleic acid encodes for a polypeptide, wherein cells infected with the said recombinant virus are capable of eliciting an immune response directly against a member selected from the group consisting of:

(1) the polypeptide;
(2) a fragment of the polypeptide;
(3) a cell expressing the polypeptide or a fragment thereof; or
(4) cells binding the protein or fragment thereof.

15. A recombinant virus according to claim 13 or 14 selected from adenovirus, alphavirus, or poxvirus.

16. A recombinant virus according to claim 15 wherein the poxvirus is vaccinia, fowlpox, avipox, TROVAC, ALVAC, NYVAC or MVA.

17. A recombinant virus according to claim 16 wherein the virus is ALVAC.

18. A composition comprising a recombinant virus of any one of claims 13 to 17 and a pharmaceutically acceptable diluent or carrier.

19. An isolated protein encoded by a nucleic acid molecule according to any one of claims 1-7.

20. An isolated protein having the activity of a modified gp100 protein.

21. A protein having the amino acid sequence shown in Figure 2 (SEQ.ID.NO.2).

22. A method of modulating an animal's immune system comprising administering an effective amount of a gp100 or *gp100* which has been modified.

23. A method according to claim 22 where the gp100 is gp100M.

24. A method according to claim 22 wherein the *gp100* is *gp100M*.

25. A method according to claim 24 wherein the gp100M has a nucleic acid sequence as shown in Figure 1 (SEQ.ID.NO.1).

26. A method according to claim 23 wherein the gp100M has an amino acid shown in Figure 2 (SEQ.ID.NO.2).

27. A method of modulating an animal's immune system comprising administering to an animal in need thereof, an effective amount of a vector, into which has been inserted a *gp100* which has been modified, thereby modulating the animal's immune system.

28. A method according to claim 27 wherein the vector is administered with a lymphokine, cytokine, or a co-stimulatory molecule.

29. A method according to claim 28 wherein the cytokine is GM-CSF, IL-2, IL-12, TNF, or IFNγ1.

30. A method according to claim 28 wherein the molecule is a lymphokine.

31. A method according to claim 28 wherein the molecule is co-stimulatory molecule.

32. A method according to claim 31 wherein the co-stimulatory molecule is a molecule of the B7 family.

33. A method according to any one of claims 27-32 wherein the vector is an adenovirus, alphavirus or poxvirus.

34. A method according to claim 33 wherein the poxvirus is vaccinia, fowlpox, avipox, TROVAC, ALVAC, NYVAC or MVA.

35. A method according to claim 34 wherein the poxvirus is ALVAC

36. A method for prophylactic treatment of cancer comprising administering to an animal an effective amount of a modified gp100 or

immunogenic fragment thereof, or a nucleic acid sequence encoding a modified *gp100* or immunogenic fragment thereof.

37. A method according to claim 36 wherein the modified gp100 has an amino acid sequence as shown in Figure 2 (SEQ.ID.NO.2).

38. A method according to claim 36 wherein the nucleic acid sequence is as shown in Figure 1 (SEQ.ID.NO.1).

39. A method according to any one of claims 36, 37 or 38 wherein the cancer is a melanoma.

40. A melanoma vaccine comprising a nucleic acid sequence encoding a modified gp100.

41. A vaccine according to claim 40 wherein the modified gp100 is gp100M.

42. A vaccine according to claim 41 wherein the gp100M has the amino acid sequence as shown in Figure 2 (SEQ.ID.NO.2).

43. A modified gp100 protein sequence which is modified to enhance its binding to MHC molecules.

44. A modified protein sequence according to claim 43 wherein the protein is gp100M.

45. The protein of claim 44 wherein the amino acid sequence is as shown in Figure 2 (SEQ.ID.NO.2).

46. A vaccine comprising a modified *gp100* nucleic acid sequence or its corresponding protein or protein fragment capable of eliciting the production of antibodies in a animal to corresponding antigens.

47. A vaccine according to claim 46 wherein the protein corresponding to the nucleic acid sequence is gp100M.

48. A vaccine according to claim 46 wherein the modified *gp100* nucleic acid sequence is *gp100M*.

49. A vaccine according to claim 48 wherein the *gp100M* nucleic acid sequence is as shown in Figure 1 (SEQ.ID.NO.1).

50. A vaccine according to claim 47 wherein the gp100M has an amino acid sequence as shown in Figure 2 (SEQ.ID.NO.2).

51. A vaccine comprising a modified gp100 nucleic acid sequence or its corresponding protein or protein fragment capable of eliciting a cellular immune response.

52. A vaccine according to claim 51 wherein the protein corresponding to the nucleic acid sequence is gp100M.

53. A vaccine according to claim 51 wherein the modified *gp100* nucleic acid sequence is *gp100M*.

54. A vaccine according to claim 53 wherein the gp100M nucleic acid sequence is as shown in Figure 1 (SEQ.ID.NO.1).

55. A vaccine according to claim 52 wherein the gp100M has an amino acid sequence is as shown in Figure 2 (SEQ.ID.NO.2).

56. An immunogenic composition containing a vaccine vector encoding for a modified gp100 molecule.

57. A composition according to claim 56 wherein the modified gp100 molecule is gp100M.

58. A composition according to claim 57 wherein the modified gp100M has an amino acid sequence according to Figure 2 (SEQ.ID.NO.2).

59. A composition according to any one of claims 56, 57 or 58 wherein the vector is an adenovirus, alphavirus or poxvirus.

60. A composition according to claim 59 wherein the poxvirus is vaccinia, fowlpox, avipox, TROVAC, ALVAC, NYVAC or MVA.

61. A composition according to claim 60 wherein the poxvirus is ALVAC.

62. Immunogenic fragments of an isolated gp100M protein encoded by a nucleic acid molecule having a sequence according to SED ID NO. 1.

FIGURE 1

```
          ATGG ATCTGGTGCT AAAAAGATGC CTTCTTCATT TGGCTGTGAT
AGGTGCTTTG CTGGCTGTGG GGGCTACAAA AGTACCCAGA AACCAGGACT GGCTTGGTGT
CTCAAGGCAA CTCAGAACCA AAGCCTGGAA CAGGCAGCTG TATCCAGAGT GGACAGAAGC
CCAGAGACTT GACTGCTGGA GAGGTGGTCA AGTGTCCCTC AAGGTCAGTA ATGATGGGCC
TACACTGATT GGTGCAAATG CCTCCTTCTC TATTGCCTTG AACTTCCCTG GAAGCCAAAA
GGTATTGCCA GATGGGCAGG TTATCTGGGT CAACAATACC ATCATCAATG GGAGCCAGGT
GTGGGGAGGA CAGCCAGTGT ATCCCCAGGA AACTGACGAT GCCTGCATCT TCCCTGATGG
TGGACCTTGC CCATCTGGCT CTTGGTCTCA GAAGAGAAGC TTTGTTTATG TCTGGAAGAC
CTGGGGCCAA TACTGGCAAG TTCTAGGGGG CCCAGTGTCT GGGCTGAGCA TTGGGACAGG
CAGGGCAATG CTGGGCACAC ACACGATGGA AGTGACTGTC TACCATCGCC GGGGATCCCG
GAGCTATGTG CCTCTTGCTC ATTCCAGCTC AGCCTTCACC ATTATGGACC AGGTGCCTTT
CTCCGTGAGC GTGTCCCAGT TGCGGGCCTT GGATGGAGGG AACAAGCACT TCCTGAGAAA
TCAGCCTCTG ACCTTTGCCC TCCAGCTCCA TGACCCCAGT GGCTATCTGG CTGAAGCTGA
CCTCTCCTAC ACCTGGGACT TTGGAGACAG TAGTGGAACC CTGATCTCTC GGGCACTTGT
GGTCACTCAT ACTTACCTGG AGCCTGGCCC AGTCACTGTT CAGGTGGTCC TGCAGGCTGC
CATTCCTCTC ACCTCCTGTG GCTCCTCCCC AGTTCCAGGC ACCACAGATG GGCACAGGCC
AACTGCAGAG GCCCCTAACA CCACAGCTGG CCAAGTGCCT ACTACAGAAG TTGTGGGTAC
TACACCTGGT CAGGCGCCAA CTGCAGAGCC CTCTGGAACC ACATCTGTGC AGGTGCCAAC
CACTGAAGTC ATAAGCACTG CACCTGTGCA GATGCCAACT GCAGAGAGCA CAGGTATGAC
ACCTGAGAAG GTGCCAGTTT CAGAGGTCAT GGGTACCACA CTGGCAGAGA TGTCAACTCC
AGAGGCTACA GGTATGACAC CTGCAGAGGT ATCAATTGTG GTGCTTTCTG GAACCACAGC
TGCACAGGTA ACAACTACAG AGTGGGTGGA GACCACAGCT AGAGAGCTAC CTATCCCTGA
GCCTGAAGGT CCAGATGCCA GCTCAATCAT GTCTACGGAA AGTATTACAG GTTCCCTGGG
CCCCCTGCTG GATGGTACAG CCACCTTAAG GCTGGTGAAG AGACAAGTCC CCCTGGATTG
TGTTCTGTAT CGATATGGTT CCTTTTCCGT CACCCTGGAC ATTGTCCAGG GTATTGAAAG
TGCCGAGATC CTGCAGGCTG TGCCGTCCGG TGAGGGGGAT GCATTTGAGC TGACTGTGTC
CTGCCAAGGC GGGCTGCCCA AGGAAGCCTG CATGGAGATC TCATCGCCAG GGTGCCAGCC
CCCTGCCCAG CGGCTGTGCC AGCCTGTGCT ACCCAGCCCA GCCTGCCAGC TGGTTCTGCA
CCAGATACTG AAGGGTGGCT CGGGGACATA CTGCCTCAAT GTGTCTCTGG CTGATACCAA
CAGCCTGGCA GTGGTCAGCA CCCAGCTTAT CATGCCTGGT CAAGAAGCAG GCCTTGGGCA
GGTTCCGCTG ATCGTGGGCA TCTTGCTGGT GTTGATGGCT GTGGTCCTTG CATCTCTGAT
ATATAGGCGC AGACTTATGA AGCAAGACTT CTCCGTACCC CAGTTGCCAC ATAGCAGCAG
TCACTGGCTG CGTCTACCCC GCATCTTCTG CTCTTGTCCC ATTGGTGAGA ACAGCCCCCT
CCTCAGTGGG CAGCAGGTCT GA
```

FIGURE 2

```
Met Asp Leu Val Leu Lys Arg Cys Leu Leu His Leu Ala Val Ile Gly
  1               5                  10                  15
Ala Leu Leu Ala Val Gly Ala Thr Lys Val Pro Arg Asn Gln Asp Trp
            20                  25                  30
Leu Gly Val Ser Arg Gln Leu Arg Thr Lys Ala Trp Asn Arg Gln Leu
        35                  40                  45
Tyr Pro Glu Trp Thr Glu Ala Gln Arg Leu Asp Cys Trp Arg Gly Gly
    50                  55                  60
Gln Val Ser Leu Lys Val Ser Asn Asp Gly Pro Thr Leu Ile Gly Ala
65                  70                  75                  80
Asn Ala Ser Phe Ser Ile Ala Leu Asn Phe Pro Gly Ser Gln Lys Val
                85                  90                  95
Leu Pro Asp Gly Gln Val Ile Trp Val Asn Asn Thr Ile Ile Asn Gly
            100                 105                 110
Ser Gln Val Trp Gly Gly Gln Pro Val Tyr Pro Gln Glu Thr Asp Asp
        115                 120                 125
Ala Cys Ile Phe Pro Asp Gly Gly Pro Cys Pro Ser Gly Ser Trp Ser
    130                 135                 140
Gln Lys Arg Ser Phe Val Tyr Val Trp Lys Thr Trp Gly Gln Tyr Trp
145                 150                 155                 160
Gln Val Leu Gly Gly Pro Val Ser Gly Leu Ser Ile Gly Thr Gly Arg
                165                 170                 175
Ala Met Leu Gly Thr His Thr Met Glu Val Thr Val Tyr His Arg Arg
            180                 185                 190
Gly Ser Arg Ser Tyr Val Pro Leu Ala His Ser Ser Ser Ala Phe Thr
        195                 200                 205
Ile Met Asp Gln Val Pro Phe Ser Val Ser Val Ser Gln Leu Arg Ala
    210                 215                 220
Leu Asp Gly Gly Asn Lys His Phe Leu Arg Asn Gln Pro Leu Thr Phe
225                 230                 235                 240
Ala Leu Gln Leu His Asp Pro Ser Gly Tyr Leu Ala Glu Ala Asp Leu
                245                 250                 255
Ser Tyr Thr Trp Asp Phe Gly Asp Ser Ser Gly Thr Leu Ile Ser Arg
            260                 265                 270
Ala Leu Val Val Thr His Thr Tyr Leu Glu Pro Gly Pro Val Thr Val
        275                 280                 285
Gln Val Val Leu Gln Ala Ala Ile Pro Leu Thr Ser Cys Gly Ser Ser
    290                 295                 300
Pro Val Pro Gly Thr Thr Asp Gly His Arg Pro Thr Ala Glu Ala Pro
305                 310                 315                 320
Asn Thr Thr Ala Gly Gln Val Pro Thr Thr Glu Val Val Gly Thr Thr
                325                 330                 335
Pro Gly Gln Ala Pro Thr Ala Glu Pro Ser Gly Thr Thr Ser Val Gln
            340                 345                 350
Val Pro Thr Thr Glu Val Ile Ser Thr Ala Pro Val Gln Met Pro Thr
        355                 360                 365
```

FIGURE 2 (CONT'D)

```
Ala Glu Ser Thr Gly Met Thr Pro Glu Lys Val Pro Val Ser Glu Val
    370                 375                 380
Met Gly Thr Thr Leu Ala Glu Met Ser Thr Pro Glu Ala Thr Gly Met
385                 390                 395                 400
Thr Pro Ala Glu Val Ser Ile Val Val Leu Ser Gly Thr Thr Ala Ala
                405                 410                 415
Gln Val Thr Thr Thr Glu Trp Val Glu Thr Thr Ala Arg Glu Leu Pro
            420                 425                 430
Ile Pro Glu Pro Glu Gly Pro Asp Ala Ser Ser Ile Met Ser Thr Glu
        435                 440                 445
Ser Ile Thr Gly Ser Leu Gly Pro Leu Leu Asp Gly Thr Ala Thr Leu
    450                 455                 460
Arg Leu Val Lys Arg Gln Val Pro Leu Asp Cys Val Leu Tyr Arg Tyr
465                 470                 475                 480
Gly Ser Phe Ser Val Thr Leu Asp Ile Val Gln Gly Ile Glu Ser Ala
                485                 490                 495
Glu Ile Leu Gln Ala Val Pro Ser Gly Glu Gly Asp Ala Phe Glu Leu
            500                 505                 510
Thr Val Ser Cys Gln Gly Gly Leu Pro Lys Glu Ala Cys Met Glu Ile
        515                 520                 525
Ser Ser Pro Gly Cys Gln Pro Pro Ala Gln Arg Leu Cys Gln Pro Val
    530                 535                 540
Leu Pro Ser Pro Ala Cys Gln Leu Val Leu His Gln Ile Leu Lys Gly
545                 550                 555                 560
Gly Ser Gly Thr Tyr Cys Leu Asn Val Ser Leu Ala Asp Thr Asn Ser
                565                 570                 575
Leu Ala Val Val Ser Thr Gln Leu Ile Met Pro Gly Gln Glu Ala Gly
            580                 585                 590
Leu Gly Gln Val Pro Leu Ile Val Gly Ile Leu Leu Val Leu Met Ala
        595                 600                 605
Val Val Leu Ala Ser Leu Ile Tyr Arg Arg Arg Leu Met Lys Gln Asp
    610                 615                 620
Phe Ser Val Pro Gln Leu Pro His Ser Ser Ser His Trp Leu Arg Leu
625                 630                 635                 640
Pro Arg Ile Phe Cys Ser Cys Pro Ile Gly Glu Asn Ser Pro Leu Leu
                645                 650                 655
Ser Gly Gln Gln Val
            660
```

FIGURE 3

Nucleotide Sequence of C5H6gp100M

```
1-254        left C5 flanking arm
255-376      H6 promoter
377-2362     modified gp100 gene
2363-2534    right C5 flanking arm
```

```
   1 GGCTACTTTT CAACAAAGGA GCAGATGTAA ACTACATCTT TGAAAGAAAT GGAAAATCAT
  61 ATACTGTTTT GGAATTGATT AAAGAAAGTT ACTCTGAGAC ACAAAAGAGG TAGCTGAAGT
 121 GGTACTCTCA AAGGTACGTG ACTAATTAGC TATAAAAAGG ATCGTCGACG AGCTCGAATT
 181 CGGATCCGGG TTAATTAATT AGTCATCAGG CAGGGCGAGA ACGAGACTAT CTGCTCGTTA
 241 ATTAATTAGA GCTTCTTTAT TCTATACTTA AAAAGTGAAA ATAAATACAA AGGTTCTTGA
 301 GGGTTGTGTT AAATTGAAAG CGAGAAATAA TCATAAATTA TTTCATTATC GCGATATCCG
 361 TTAAGTTTGT ATCGTAATGG ATCTGGTGCT AAAAAGATGC CTTCTTCATT TGGCTGTGAT
 421 AGGTGCTTTG CTGGCTGTGG GGGCTACAAA AGTACCCAGA AACCAGGACT GGCTTGGTGT
 481 CTCAAGGCAA CTCAGAACCA AAGCCTGGAA CAGGCAGCTG TATCCAGAGT GGACAGAAGC
 541 CCAGAGACTT GACTGCTGGA GAGGTGGTCA AGTGTCCCTC AAGGTCAGTA ATGATGGGCC
 601 TACACTGATT GGTGCAAATG CCTCCTTCTC TATTGCCTTG AACTTCCCTG GAAGCCAAAA
 661 GGTATTGCCA GATGGGCAGG TTATCTGGGT CAACAATACC ATCATCAATG GGAGCCAGGT
 721 GTGGGGAGGA CAGCCAGTGT ATCCCCAGGA AACTGACGAT GCCTGCATCT TCCCTGATGG
 781 TGGACCTTGC CCATCTGGCT CTTGGTCTCA GAAGAGAAGC TTTGTTTATG TCTGGAAGAC
.841 CTGGGGCCAA TACTGGCAAG TTCTAGGGGG CCCAGTGTCT GGGCTGAGCA TTGGGACAGG
 901 CAGGGCAATG CTGGGCACAC ACACGATGGA AGTGACTGTC TACCATCGCC GGGGATCCCG
 961 GAGCTATGTG CCTCTTGCTC ATTCCAGCTC AGCCTTCACC ATTATGGACC AGGTGCCTTT
1021 CTCCGTGAGC GTGTCCCAGT TGCGGGCCTT GGATGGAGGG AACAAGCACT TCCTGAGAAA
1081 TCAGCCTCTG ACCTTTGCCC TCCAGCTCCA TGACCCCAGT GGCTATCTGG CTGAAGCTGA
1141 CCTCTCCTAC ACCTGGGACT TTGGAGACAG TAGTGGAACC CTGATCTCTC GGGCACTTGT
1201 GGTCACTCAT ACTTACCTGG AGCCTGGCCC AGTCACTGTT CAGGTGGTCC TGCAGGCTGC
1261 CATTCCTCTC ACCTCCTGTG GCTCCTCCCC AGTTCCAGGC ACCACAGATG GGCACAGGCC
1321 AACTGCAGAG GCCCCTAACA CCACAGCTGG CCAAGTGCCT ACTACAGAAG TTGTGGGTAC
1381 TACACCTGGT CAGGCGCCAA CTGCAGAGCC CTCTGGAACC ACATCTGTGC AGGTGCCAAC
1441 CACTGAAGTC ATAAGCACTG CACCTGTGCA GATGCCAACT GCAGAGAGCA CAGGTATGAC
1501 ACCTGAGAAG GTGCCAGTTT CAGAGGTCAT GGGTACCACA CTGGCAGAGA TGTCAACTCC
1561 AGAGGCTACA GGTATGACAC CTGCAGAGGT ATCAATTGTG GTGCTTTCTG GAACCACAGC
1621 TGCACAGGTA ACAACTACAG AGTGGGTGGA GACCACAGCT AGAGAGCTAC CTATCCCTGA
1681 GCCTGAAGGT CCAGATGCCA GCTCAATCAT GTCTACGGAA AGTATTACAG GTTCCCTGGG
1741 CCCCCTGCTG GATGGTACAG CCACCTTAAG GCTGGTGAAG AGACAAGTCC CCCTGGATTG
1801 TGTTCTGTAT CGATATGGTT CCTTTTCCGT CACCCTGGAC ATTGTCCAGG GTATTGAAAG
1861 TGCCGAGATC CTGCAGGCTG TGCCGTCCGG TGAGGGGGAT GCATTTGAGC TGACTGTGTC
1921 CTGCCAAGGC GGGCTGCCCA AGGAAGCCTG CATGGAGATC TCATCGCCAG GGTGCCAGCC
1981 CCCTGCCCAG CGGCTGTGCC AGCCTGTGCT ACCCAGCCCA GCCTGCCAGC TGGTTCTGCA
2041 CCAGATACTG AAGGGTGGCT CGGGGACATA CTGCCTCAAT GTGTCTCTGG CTGATACCAA
2101 CAGCCTGGCA GTGGTCAGCA CCCAGCTTAT CATGCCTGGT CAAGAAGCAG GCCTTGGGCA
2161 GGTTCCGCTG ATCGTGGGCA TCTTGCTGGT GTTGATGGCT GTGGTCCTTG CATCTCTGAT
2221 ATATAGGCGC AGACTTATGA AGCAAGACTT CTCCGTACCC CAGTTGCCAC ATAGCAGCAG
2281 TCACTGGCTG CGTCTACCCC GCATCTTCTG CTCTTGTCCC ATTGGTGAGA ACAGCCCCCT
2341 CCTCAGTGGG CAGCAGGTCT GATTTTTATC TCGAGTCTAG AATCGATCCC GGGTTTTTAT
2401 GACTAGTTAA TCACGGCCGC TTATAAAGAT CTAAAATGCA TAATTTCTAA ATAATGAAAA
2461 AAAAGTACAT CATGAGCAAC GCGTTAGTAT ATTTTACAAT GGAGATTAAC GCTCTATACC
2521 GTTCTATGTT TATT
```

FIGURE 4

ALVAC(2)-gp100M (vCP1584)

(ALVAC *Xho*I Restriction Map)

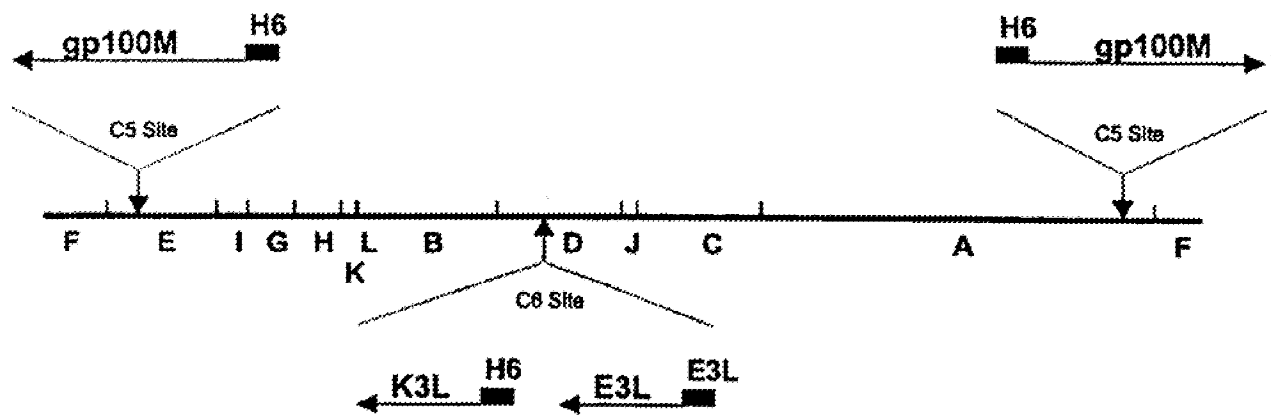


FIGURE 5

Oligonucleotide Primers

IDC5-1
CGT GCC ATG GCA CAC AAA AGA GGT AGC TGA A

IDC5-2
CCA GGC GGC CGC ACT AAC GCG TTG CTC ATG ATG

C5L
CAC AAA AGA GGT AGC TGA AGT

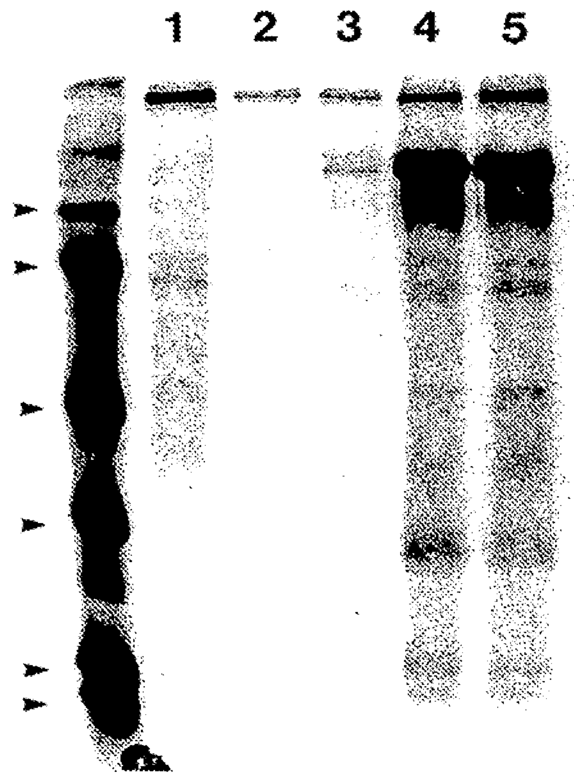
MEL 01
ATG GAT CTG GTG CTA AAA AGA

MEL 05
ACC TTG CCC ATC TGG CTC TTG

MEL 09
AGA TGC CAG CTC AAT CAT GTG

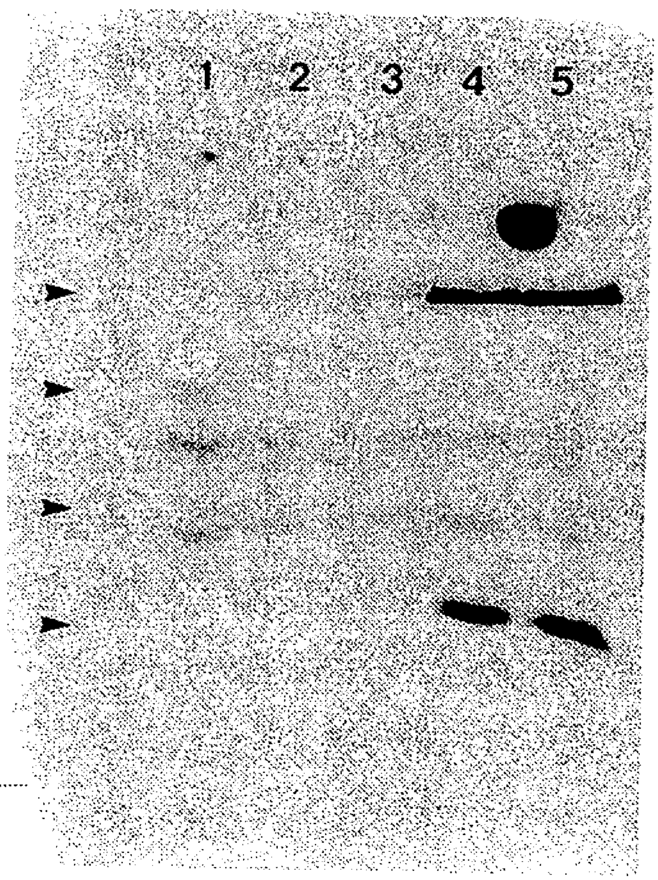
C5R
ATA GAT CTT TAT AAG CGG CCG

FIGURE 6



Molecular Weight Markers: 200, 98.6, 68, 43, 29, 18, 14 kDa
Lane 1: Uninfected HeLa cells
Lane 2: HeLa cells infected with ALVAC
Lane 3: HeLa cells infected with ALVAC-gp100 (vCP1465)
Lane 4: HeLa cells infected with ALVAC(2)-gp100M (vCP1584)
Lane 5: HeLa cells infected with ALVAC(2)-gp100M (sister of vCP1584)

FIGURE 7



Molecular Weight Markers: 97, 68, 43, 29 kDa
Lane 1: Uninfected HeLa cells
Lane 2: HeLa cells infected with ALVAC
Lane 3: HeLa cells infected with ALVAC-gp100 (vCP1465)
Lane 4: HeLa cells infected with ALVAC(2)-gp100M (vCP1584)

FIGURE 8

Monkey #6 (Intranodal Administration)

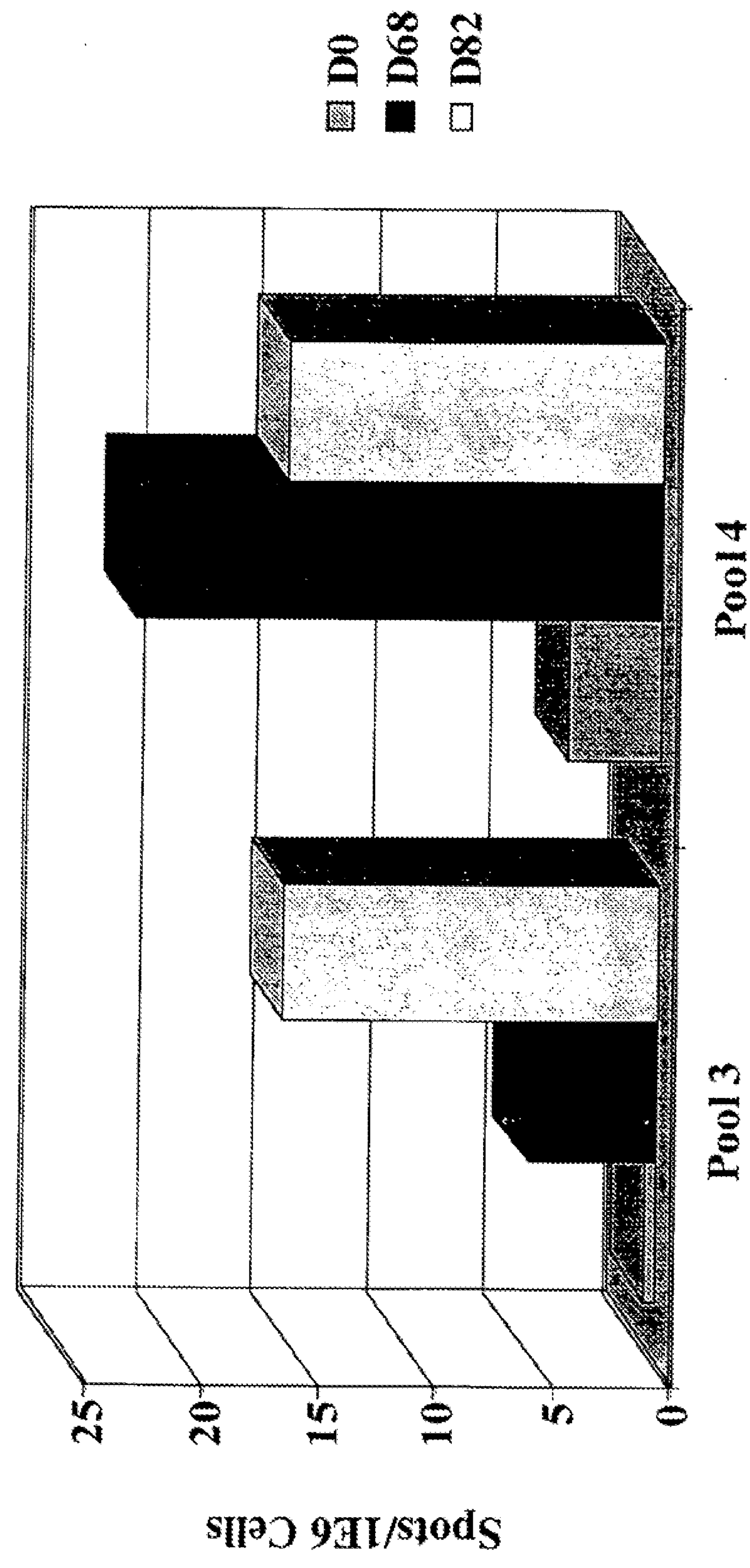


FIGURE 9

Monkey #7 (Intranodal Administration)

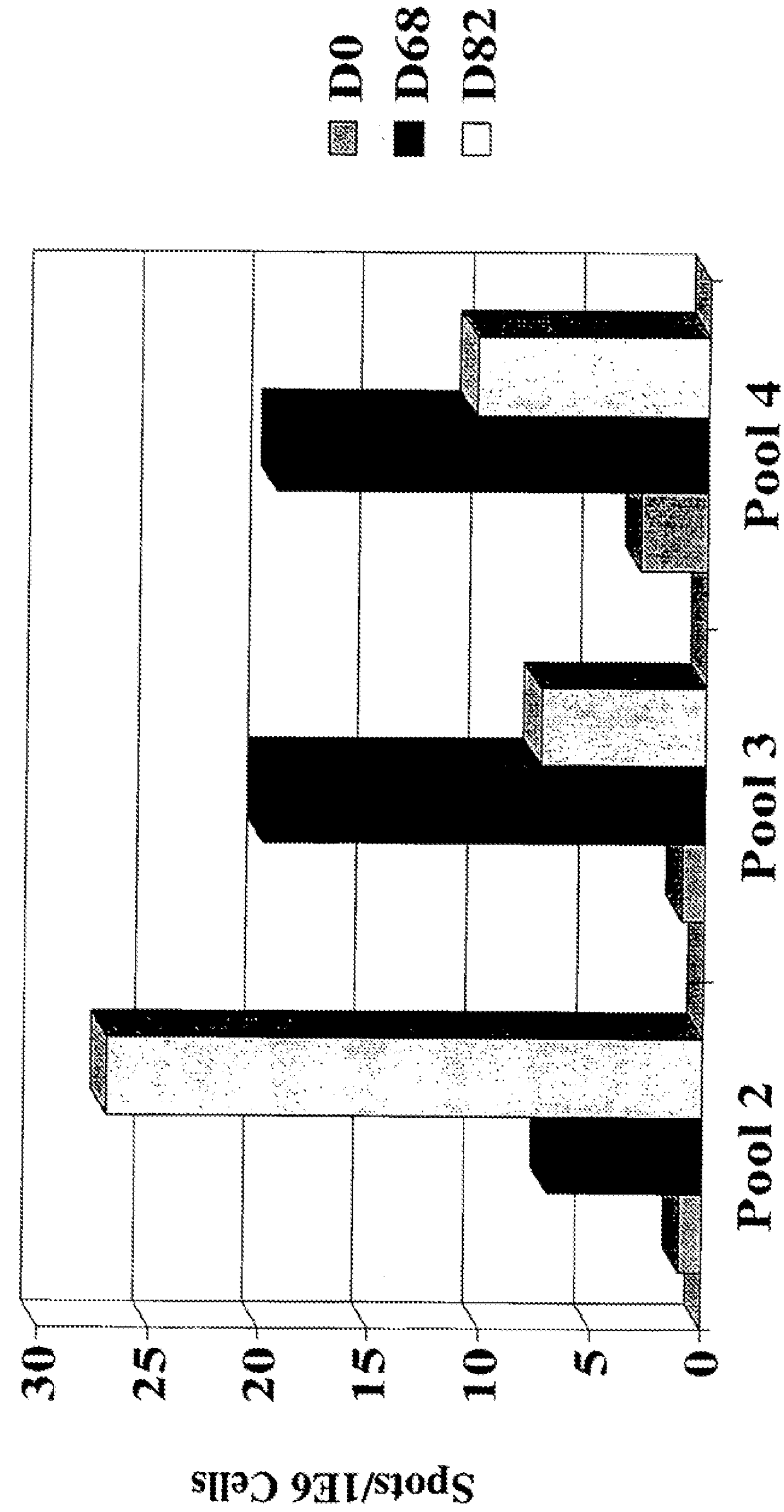


FIGURE 10

Monkey # 11 (Subcutaneous Administration)

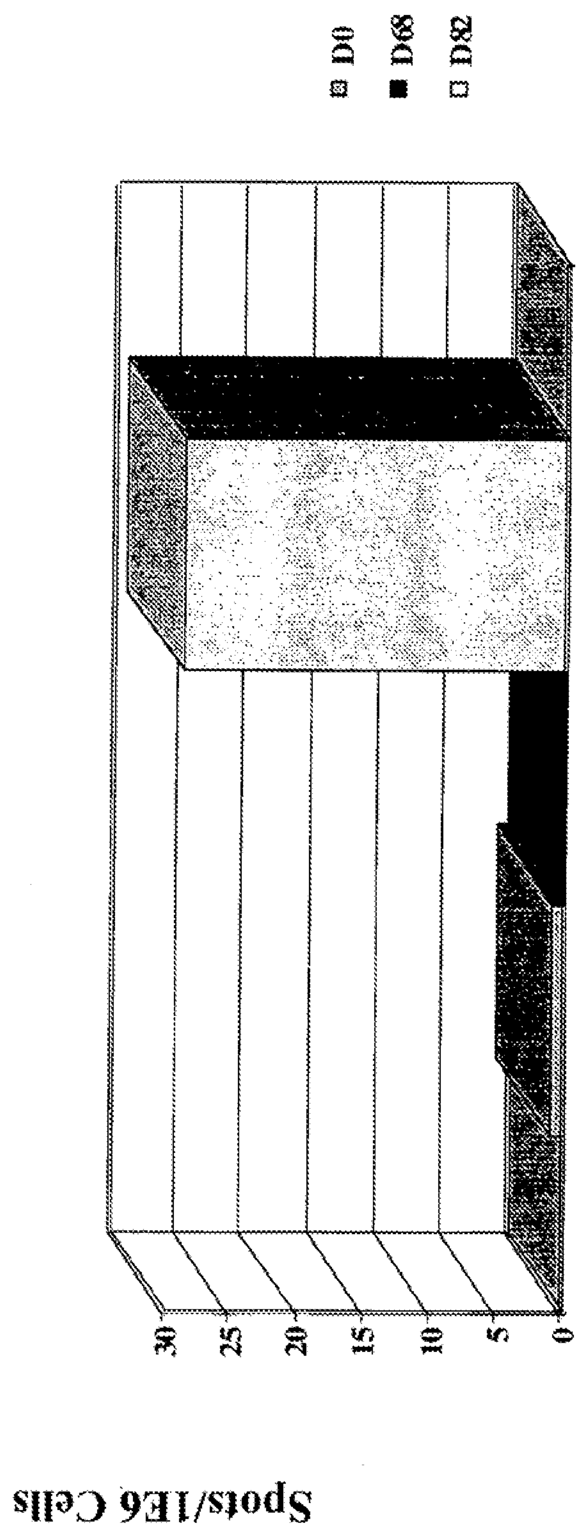
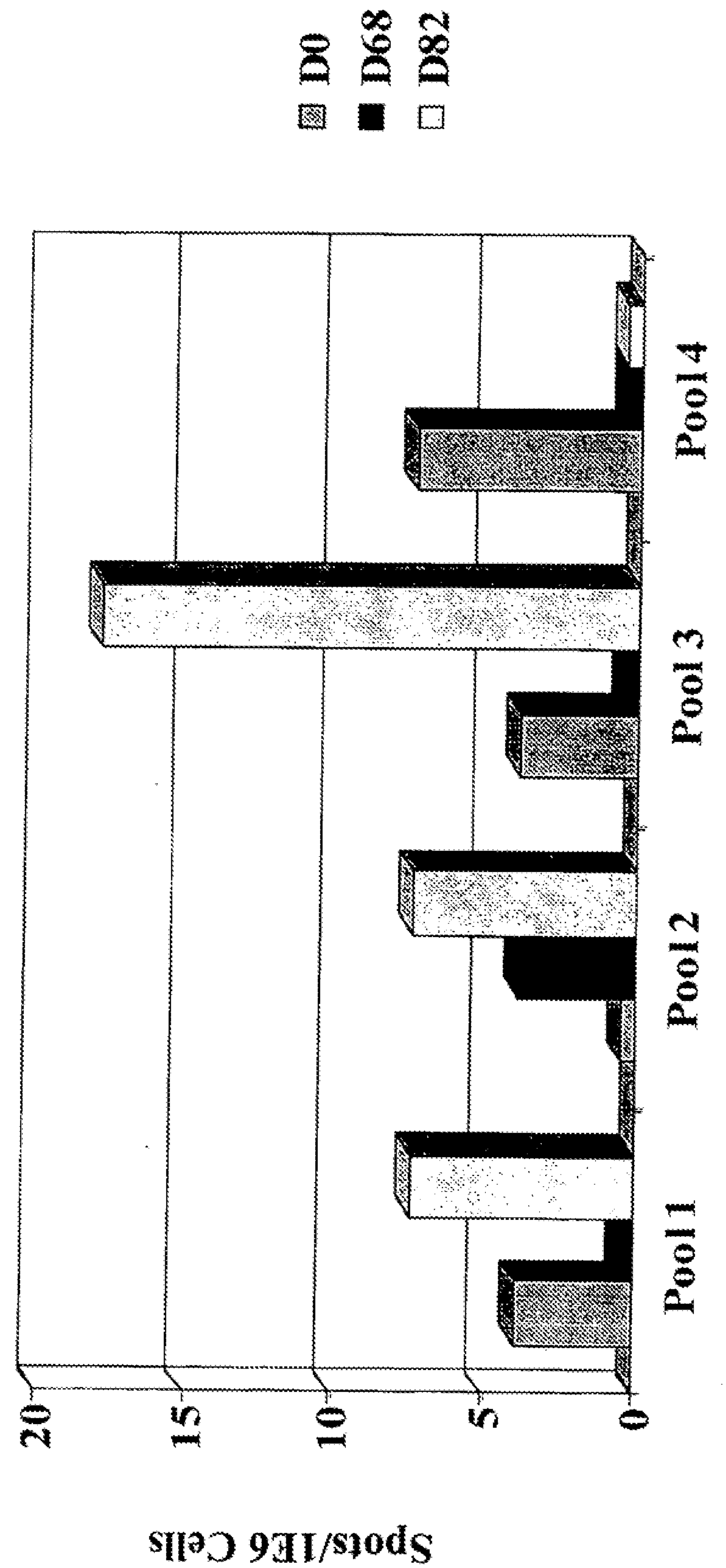


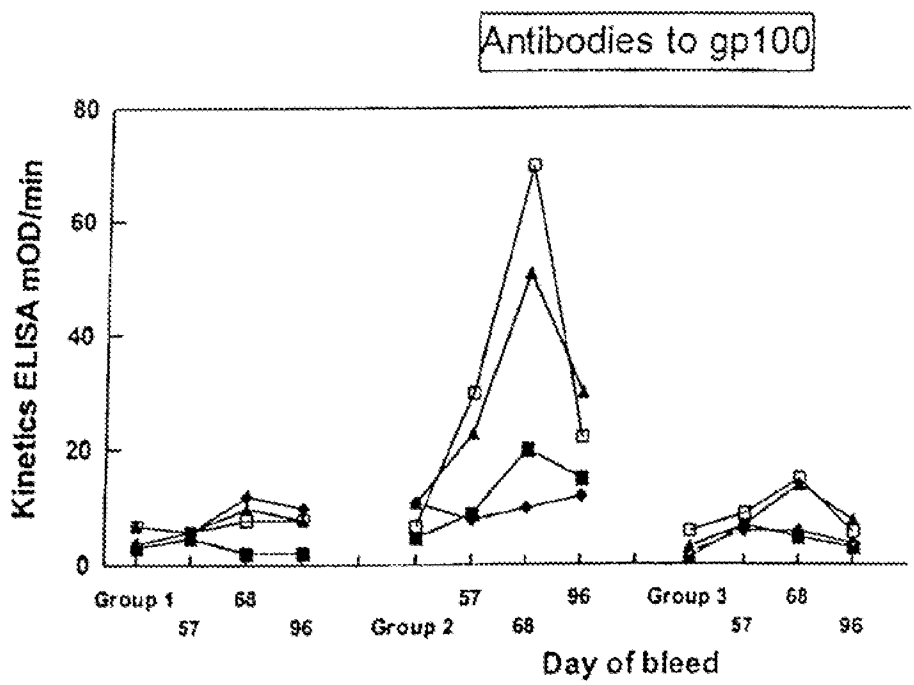
FIGURE 11

Monkey #10 (Subcutaneous Administration)



13/13

FIGURE 12



INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 00/01254

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/705 C12N9/64 A61K39/00 C12N5/06 C12N7/00
A61K35/76 A61P35/00 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

MEDLINE, CANCERLIT, LIFESCIENCES, EMBASE, CHEM ABS Data, SCISEARCH, BIOSIS, WPI Data, EPO-Internal, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	IRVINE KARI R ET AL: "Recombinant virus vaccination against "self" antigens using anchor-fixed immunogens." CANCER RESEARCH, vol. 59, no. 11, 1 June 1999 (1999-06-01), pages 2536-2540, XP002161590 ISSN: 0008-5472 page 2536, left-hand column, line 39 -right-hand column, line 14 page 2356, right-hand column, line 47 -page 2357, left-hand column, line 1 table 1 -----	8,9, 13-16, 18-20, 22-24, 27-34, 36, 39-41, 43,44, 46-48, 51-53, 56,57, 59,60

-/--

[X] Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search	Date of mailing of the international search report
28 February 2001	12/03/2001

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